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Preliminary Results of Wood Endophytes of *Abies cilicica* in Yenişarbademli of Isparta Province

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Abstract
Fungal colonisation originating from endophytic thalli in wood of healthy Taurus fir (*Abies cilicica*) was studied. Bore cores were taken from living trees during field surveys. The bore cores were cut into 0.5 cm long fragments, washed with 70% (v/v) ethanol, flamed and placed into petri dishes containing malt extract, agar and streptomycin. The plates were incubated at 25°C for 4 weeks. The outgrowing mycelia were pure-cultured and identified based on amplification and sequencing of the internal transcribed spacer (ITS) region of their nuclear ribosomal DNA. Totally 12 different fungal species were isolated from *Abies cilicica* wood cores. Five of them belong to Basidiomycetes. The present study is the first investigation of endophytic wood fungi occurring in fir in Turkey using DNA-based methods. Therefore the study is expected to provide new information of the pioneering colonizers of wood tissues in stems of living Taurus firs.
Key words: *Abies cilicica*, wood tissue, endophyte, Basidiomycetes

Introduction

Endophytic fungi are defined as micro-organisms that colonize healthy plant tissues inter- and/or intra-cellularly, persisting for the whole or part of their life cycle without causing disease symptoms in the host plant (Petrini, 1996; Oses et al., 2008). Some of these fungi are opportunists waiting for their host to senesce at which time they can begin the decomposition of cell wall materials. The niches occupied by fungal endophytes in different plant tissues such as leaves, petioles, seeds, cones, bark and especially sapwood deserves more attention (Petrini, 1991). There is a need to further explore the role of wood-inhabiting endophytes, especially in natural processes such as wood biodegradation (Dix and Webster, 1995; Norden et al., 1999).

The aim of this study was to provide information about the natural occurrence and frequency of endophytic wood fungi in sapwood of living *A. cilicica* trees.

Material and Methods

The study was conducted in 2012 in seven *Abies cilicica* stands throughout Lake District Region of Turkey, including Konya, Isparta and Burdur provinces. A total of 103 *A. cilicica* trees (11 to 18 living trees per site) were sampled during the present study (Table 1).

Trees were investigated for decay by increment borer in 25x50 m^2 sample plots systematically chosen in each stand. The height, diameter and age of the trees in each stand were recorded.

Table 1. Sampling areas and number of trees sampled

Province	Number of stands	Number of trees sampled
Isparta /Eğirdir, Yuvalı	1	10
Isparta/Yenişarbademli	1	5
Burdur /Bucak	1	5
Konya/ Ermenek	1	5
Konya/ İslibucak	3	78
Total	7	103

Bore cores were taken from living trees during field surveys by drilling with increment borer from the two sides of stem base for each tree. The bore cores were cut into 0.5 cm long fragments, washed with 70% (v/v) ethanol, flamed and placed into Petri dishes containing malt extract (2%), agar (2%) and streptomycin (0,01gr/l). The plates were incubated at 25°C for 4 weeks. The outgrowing mycelia were pure-cultured and identified based on amplification and sequencing of the internal transcribed spacer (ITS) region of their nuclear ribosomal DNA. Each PCR reaction was performed in a 50µl volume containing 5ul of 10x PCR buffer, 5 µl of MgCl2 (25mM), 1 µl of dNTP mix (10mM), 1 µl ITS1 primer (20mM), 1 µl ITS4 primer (20mM), 0,5 µl Taq DNA polymerase and 1 µl of a DNA (50 ng). PCR reactions were conducted using an initial denaturation step at 95°C for 2 min, followed by 32 cycles consisting of a denaturation at 94°C for 20s, an annealing at a temperature of 55°C for 25s,

elongation at 72°C for 50s and an extension at 72°C for 10 min.

The sequencing of the PCR products in both directions was performed by a commercial laboratory (IonTek, Istanbul, Turkey) using the primer set ITS1 and ITS4 (White et al., 1990). The sequences were determined using an ABI PRISM automated sequencer.

The sequences were compared with known sequences in GenBank database (National Centre for Biotechnology Information, NCBI) using the BLAST (Basic Local Alignment Search Tool) algorithm and the putative taxa of the isolates determined. The sequences showing a similarity above 95% with the query sequence were considered.

Results and Discussion

A total of 4120 bore core fragments from 206 cores taken from the 103 living *A. cilicica* trees were analyzed.

Fungal growth resulted in 60% (2,472 fragments) of the plated cores, while the remaining 40% was either non-colonized or colonized by bacteria. Common moulds such as *Penicillium* spp., *Alternaria* spp., *Mucor* spp. and *Trichoderma* spp. were among the abundant taxa growing from the wood pieces. Excluding these common moulds, the number of fragments colonized by fungi was 1,479 (35.90% of all fragments). The frequency of ascomycetes and the anamorphic fungi was 49%. The total number of fungi identified to the species level was 12, five of them belonging to Basidiomycetes. Fungi isolated from the cores along with the common moulds and other sporulating species identified via their morphological characteristics and fungi identified by their ITS region sequences together with their isolation frequencies are listed in Table 2.

Heterobasidion abietinum (anamorph: *Spiniger meineckellus*) Niemelä & Korhonen, *Phellinus hartigii* Allescher & Schnabl, *Bjerkandera adusta* (Willd.) P. Karst, *Trametes trogii* Berk. (syn: *Funalia trogii*), *Fomitopsis pinicola* P. Karst, *Sarea difformis* (Fr.) Fr., *Scytalidium lignicola* Pesante and *Metarhizium flavoviride* Gams & Roszypal were the most important fungi among wood endophyte isolated from the sapwood of fir trees in this study.

H. abietinum and *P. hartigii* can directly penetrate to the host, *B. adusta*, *T. trogii* and *F. pinicola* penetrates the host mainly through the wounds. The ascomycete fungi detected in living fir trees in this study includes potential bio-control agents against wood decay fungi (*S. lignicola*) with antagonistic abilities or entomopathogenic properties against bark beetles (*Metarhizium flavoviride*).

H. abietinum, a common root and butt rot pathogen, was the most abundantly (21%) isolated fungus from the living fir trees. 12.6% of the sampled trees were infected with *H. abietinum*. Another important root rot fungus, *Phellinus hartigii* (4.7%) was isolated from 3 living trees (3%). *H. abietinum* was isolated from the trees sampled from İslibucak (Konya) and Bucak (Burdur). The presence of *H. abietinum* in living trees in these stands was already known (Lehtijärvi et al. 2010). In contrast, mycelium of *P. hartigii* was detected for the first time in living *A. cilicica* trees in Turkey. On the other hand, the fruit bodies of the fungus were observed previously in Lake District of Turkey on stumps and living conifers (Lehtijärvi et al 2008). In the present work the fungus was isolated only from one stand in Eğirdir, Yuvalı.

F. pinicola, *B. adusta* and *T. trogii*, which are common and well-known wood decay fungi, were also isolated from 10 different sample trees at different localities. However their isolation frequencies were not as high as *H. abietinum* (4.5, 4.7 and 5.9 respectively). Wood decay development largely depends on the colonization strategy adopted by the decay fungus. Heart rot, unspecialized opportunism, specialized opportunism, active pathogenesis, and desiccation tolerance are the known strategies of the wood decay fungi growing in living trees (Rayner and Boddy, 1988). For example, *F. pinicola* colonize the inner core of the tree (heartwood or ripe wood) through wounded roots or branches which expose heartwood, where living cells are absent or rare. In contrast, *T. trogii* as unspecialized opportunists can colonize the fresh sapwood of standing trees after wounding or by taking advantage of a physiological stress such as root damage or drought (Rayner and Boddy, 1988).

Table 2. Fungi isolated from the bore core fragments and their isolation frequencies

Fungi	Number of colonized fragments	Isolation frequency (%)	Nucleotide Sequence similarity (%)
Basidiomycota	**1241**	**50,20**	
Heterobasidion abietinum	519	21,00	n.s.*
Phellinus hartigii	116	4,69	n.s
Bjerkandera adusta	112	4,53	100
Trametes trogii	145	5,87	99
Fomitopsis pinicola	118	4,77	99
Polyporales sp.	203	8,21	<95
Undentified basidiomycete1	28	1,13	<85
Ascomycota and anamorphic fungi	**1085**	**43,89**	
Sarea difformis	27	1,09	99
Scytalidium lignicola	58	2,35	99
Metarhizium flavoviride	56	2,27	97-99
Epicoccum nigrum Link	85	3,44	n.s.
Alternaria spp.	44	1,78	n.s.
Aspergillus spp.	161	6,51	n.s.
Fusarium sp.	85	3,44	n.s.
Penicillium spp.	312	12,62	n.s.
Trichoderma spp.	121	4,89	n.s.
Cladosporium spp.	39	1,58	n.s
Dark sterile mycelia (unidentified ascomycetes)	51	2,06	<85
Hyaline sterile mycelia (unidentified ascomycetes)	46	1,86	<85
Zygomycota	**146**	**5,91**	
Mucor hiemalis	40	1,62	n.s.
Mucor plumbeus	35	1,42	n.s.
Rhizopus stolonifer	61	2,47	n.s.
unidentified zygomycetes	10	0,40	n.s.
	2472	**100,00**	

* n.s.: not sequenced

Besides being a white rot fungus, *T. trogii*, on the other hand, is a very important medical fungus. Extracts of the fungus have been proved to have promising anti-tumour properties. Moreover, the fungus has a traditional usage because of its protective effects for heart health of humans. The fungus is a common one reported from many parts of Turkey. However, previously it was not reported from a living tree. Also no other studies report its presence in a living tree. The fungus can be accidentally isolated from the sapwood. But where ever it was isolated, our findings clearly indicate the presence of this fungus in İslibucak.

S. lignicola is an ascomycete with antagonistic abilities against some white and brown rot wood decay fungi. Its antagonistic effect does not involve toxins. However, the fungus can overgrow other fungi (Higley, 1990). The fungus was isolated from 3 living *A. cilicica* trees (isolation frequency 2.4%).

Another ascomycete, *S. difformis*, which was observed on 27 wood fragments from 2 sample trees, one from İslibucak and one from Yuvalı sampling areas is a lignicolous, saprotrophic fungus growing on resin exudates of mainly pine species. The fungus was reported by Roll-Hansen and Roll-Hansen (1980), from stem wounds of *Picea abies*.

This study demonstrated the presence of some basidiomycetes, such as *B. adusta*, *F. pinicola* and *T. trogii*, in living *A. cilicica* stems. These species are known as saprophytes or decomposers of dead wood. On the other hand, *B. adusta* was reported from living stems of *Picea abies* as an occasional butt rot agent of *P. abies* from Germany and Latvia (Pechmann et al. 1973, Arhipova et al. 2011). Nevertheless, our findings also provided evidence that supports the latent infection hypothesis proposed by Boddy and Rayner (1983). Similar findings have been reported for alder (Fisher and Petrini, 1990), beech and aspen (Chapela, 1989).

Some fungi, including especially *T. trogii* and *S. difformis*, which are basically not related with the living sapwood of trees, can actually be invaders of dead tissues close to wounds. The fragments from bore core samples were not successively placed onto the plates; therefore, when a fungus grew from a fragment, it was not known whether the origin of the core fragment was close to bark or heartwood.

The ecological roles of endophytes are only gradually being elucidated (Arnold et al., 2009). So far, it is known that endophytes are neutral inhabitants, parasites or mutualists of their hosts. The high diversity of endophytes harboured by a single host species probably includes species with the capacity to either play each of these roles or change roles over time or under certain conditions (Arnold 2008). It is also probable that important ecological roles are manifested with regards not only to the plant they inhabit, but also to the surrounding plant community.

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